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**Performance analysis of multiple simultaneous communications
in bacterial nanonetworks**

Master of Science Thesis

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ABSTRACT

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Nanonetworks and nanocommunications are novel communication paradigms which have applications such as intra-body health monitors, targeted medicine delivery, genetic engineering, etc.

In nanonetworks messages are encoded in *Deoxyribonucleic acid (DNA)* strands and then delivered from the source to the destination, during the process the message bearing bacteria may conjugate with other free bacteria such that the messages are copied to the free ones. Single transmitter-receiver pair nanonetwork has already been studied extensively, at the mean time source/destination pairs case are nonetheless not scrutinized widely. Previous research has focused on one transmitter and one receiver scenarios, in other terms one-to-one nanonetwork communications, however such kind of scenarios are quite unrealistic in the practical sense, since in the real environment we can not assure that there is only one transmitter-receiver pair. Based on this motivation we study multiple transmitters-receivers pairs in the thesis so that more feasible data can be generated and the results that we get are more close to the real world.

In this thesis work we deploy single, multiple source/destination pair(s) simulations with different compartment size, different number of emitted/free bacteria, we collect the experiment data and analysis those data in a systematical way and plot them as figures via matlab so that the results of the experiments are visualized. Because of the randomness of the message delivering process and the process is exponentially distributed, we can use *Markov chain (MC)* as the analytical model. The model enable us to simulate various case of stochastic processes, calculate the delivery time.

Furthermore we analysis the different behaviors of the scenarios through the data and the diagrams and figure out which particular scenario boost the performance utmost. Finally we draw a series of conclusions according to the experimental data that we acquired which show the multiple transmitter-receiver pairs influence the delivery time critically.

PREFACE

First of all I want to express my gratitude to my supervisor Dmitri Moltchanov. Under his unparalleled instruction we make this work comes true. When we implemented the simulation we actually struggled with some essential matters, I once rewrote the core code of the program so that we are able to get correct results. My supervisor and I closely cooperated with each other and eventually made this work done.

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TERMS AND DEFINITIONS

TCP	Transmission Control Protocol
IP	Internet Protocol
WWW	World Wide Web
IoNT	Internet of Nano Things
IoT	Internet of Things
E.coli	Escherichia coli
CDF	Cumulative Distribution Function
pdf	Probability Distribution Function
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
FPT	first-passage time
IPT	inter-passage times
MC	Markov Chain
MCI	Markov Chain Instance
BACs	Bacterial Artificial Chromosomes
μm	micrometer
nm	nanometer
RDM	Random Direction Model
LST	Laplace-Stiltjers transform

1. INTRODUCTION

Modern networking technologies have remarkably made the world better and benefited people in many aspects. *Transmission Control Protocol (TCP)/Internet Protocol (IP)* based internet connects the computers around the planet as *World Wide Web (WWW)*, it provides services such as business, education, government, military services, email, and so on. An immense invisible web is formed via internet connection. Admittedly this web has drastically changed human's life, however, some people think that we can connect not only the meters, centimeters scale entities such as workstations, mobiles together but also we should and we could have the ability to connect nanometers scale objects so that we can construct a nanoscale communication network. In this chapter we first introduce the nanonetworks concept, then we discuss molecular nanonetworks. Third, we state the problems that we try to resolve in the thesis. Finally we present the thesis structure.

1.1 Introduction to nanonetworks

The concept of nanotechnology was first introduced by the Nobel prize winner Richard Feynman in 1959 in his talk *There's Plenty of Room at the Bottom*. His talk mainly stated the possibility of making smaller but more powerful devices in the future. The term "nanotechnology" was first mentioned in the 1970s by Norio Taniguchi [21] [22].

The atomic functional unit in nanotechnology is nanomachine, its size ranges from 1 to 100 nanometers. Nanomachine can be used to do basic calculation, sensing or actuation tasks. The performance of one single nanomachine is limited, however, with the interconnection of a bunch of nanomachines they can be organized as a more complicate system such that lots of complex tasks can be achieved. For instance, nano-robots, nano-processors and nano-memory. Eventually we can interconnect the nanonetworks with traditional internet so that *Internet of nano-things (IoNT)* [26] is implemented.

Nanomachines are arranged to be interconnected so that they are able to do more complicate jobs in the following ways:

- Nanomachines such as chemical sensors, nanoswitches etc. can not work independently. However with the cooperation and data synchronization with

networked nanomachines, they can perform very tangled duties such as in-body drug delivery and illness treatment as a whole.

- Nanomachines can be interconnected as nanonetworks. Usually nanonetworks are in distributed fashion which means there is not a central commander or centralized server in the networks.
- Sometimes nanomachines are supposed to be deployed in a large area, it is quite hard to manage these nanomachines due to the size and the volume of them. Nanonetworks can make such scenarios manageable through broadcasting and multihops communication schemes.

So now we are clear that nanonetworks is one productive and practical approach of nanotechnology which significantly improves the efficiency of single nanomachine. Also nanonetworks make the control of the distributed nanomachines possible. There are generally two scenarios of communications between nanomachines that we should implement as follows:

- Connection between nanomachines and larger electronic systems.
- Connection between two or more nanomachines.

There are various communication techniques proposed for these scenarios, such as electromagnetic, acoustic, nanomechanical or molecular. As we use molecular technology in particular our thesis work, in the coming section of this chapter we are going to introduce molecular nanonetwork.

Nanonetworks is a new communication paradigm in order to interconnect nanomachines in a cooperative way. Because of its novelty next we show the components of nanonetworks and compare it to the conventional networks.

1.1.1 Components of nanonetworks

- Transmitter: transmitter is usually a living cell, a biomedical implant or a nanorobot. Its task is that encode the messages onto molecule. Also transmitter emit the molecule either by releasing encoded molecules to the environment or attaching them to the carriers.
- Receiver: receiver detects that whether the incoming messages are the concerning messages, if so it decodes them.
- Message: in nanonetworks circumstance messages are molecules. The messages have three important properties: first, an external structure is defined in advance so that the messages can be easily recognized at the receiver side.

Second, the messages are inert, meaning that they will not be modified in the transmission environment. Finally, once they are decoded by the receiver that they can readily be eliminated.

- Medium: the medium of nanonetworks is related to the use case. For example, in a environmental monitoring application the medium can be air and water, the medium of a in-body health control application could be fluid, blood. The propagation speed is also affected by the medium.
- Carrier: molecular motors and calcium ions are used as carriers.

1.1.2 Comparison of nanonetworks and its traditional counterpart

Communication	Traditional	Molecular
Communication carrier	Electromagnetic waves	Molecules
Signal type	Electronic and optical (Electromagnetic)	Chemical
Propagation speed	Very fast(nearly light speed)	Extremely low
Medium condition	Wired: almost immune Wireless: Affect communication	Affect communication
Noise	Electromagnetic fields and signals	Particles and molecules in medium
Encoded information	Videos, text, audio	Phenomena, chemical states or processes
Other features	High energy consumption	Low energy consumption

Table 1.1: Main differences between traditional communication networks and nanonetworks enabled by molecular communication [22].

There are some principal differences between traditional computer/wireless networks and nanonetworks including (i) the communication carriers are different. In traditional networks carriers are electromagnetic waves while in molecular nanonetworks the carriers are molecules, (ii) in traditional networks electronic and optical are the signal type while chemicals are the signal type in nanonetworks, (iii) The propagation speed in traditional networks is fast almost light speed while in nanonetworks the speed is extremely low, (iv) in the internet wired medium such as coaxial cable and fibers are almost immune , mean time for wireless networks the medium condition can interfere the communication effect. In nanonetworks the medium condition has the same impact on communication as wireless networks, (v) noise in

traditional networks are electromagnetic fields and signals, in nanoscale networks particles and molecules in medium are the noise nonetheless, (vi) traditional networks encode the information such as videos, audios and text, on the other hand phenomena, chemical states or processes are encoded in nanonetworks. In addition the power consumption in the internet is high, for example the electricity consumption of cloud computing would cause a huge amount of green house gas emission. While it is low in the nanonetworks. We briefly list the differences in Table 1.1.

1.2 Molecular nanonetworks

As we have mentioned in the earlier part of this chapter that messages are taken by molecules. Also molecular motor is one of the carriers which nanonetworks can utilize. Furthermore, in this thesis work we use molecular/bacterial nanonetworks. So we stage a little more detail in molecular/bacterial nanonetworks.

Molecules are the smallest particles in chemical elements and compounds. They are made up of atoms that are held together by chemical bonds. These bonds form as a result of the sharing or exchange of electrons among atoms. One important property of molecules is that they are always moving. In a solid material, the movements of molecules look like rapid vibration, in a liquid environment they move randomly among each other [24]. This property provides us one of the foundation of our implementation - chemotaxis. The chemotaxis move from the transmitter to receiver via random trajectories inside the compartment so that the messages are delivered.

In general molecule communications are classified in three categories the type of propagation [17]:

- walkway-based molecular communication can be achieved either by molecular carriers such as molecular motor or by using bacteria like *E. coli* as chemotaxis.
- flow-based molecular communication, the molecules propagate through diffusion in a fluidic medium whose flow and turbulence are guided and predictable. For instance, the hormonal communication through blood streams inside human body is the communication in flow-based propagation manner.
- diffusion-based molecular communication, the molecules propagate through spontaneous diffusion in a fluidic medium. Calcium signaling is one of the example of this type of communication.

Not only just a research interest of the scientists but nanonetworks have already produced a lot of applications such as intra-body healthy control, disease diagnosis and *Internet of Things (IoT)*. As a crucial branch of communication networks technology nanonetworks are playing a more and more important role in the real world.

Below we show some applications of nanonetworks [19]:

- Health monitoring: nanonetworks which are composed of nanosensors are able to monitor sodium, glucose and other ions in blood, cholesterol or the presence of different infectious agents. For instance, a wireless interface between the nanosensors and a micro-device such as a cellphone or specialized medical equipment could be used to collect all these data and forward them to the healthcare provider.
- Targeted drug delivery: nanomachines which are controlled by central commander can be used for drug delivery system. For example, releasing a specific drug in unreachable locations of our body. At the mean time the data of human body are collected and sent back to some remote doctors or some health service persons.
- Plant monitoring system: trees, herbs, or bushes, release several chemical composites to the air in order to attract the natural predators of the insects which are attacking them, or to regulate their blooming among different plantations, amongst others. Chemical nanosensors can be used to detect the chemical compounds that are being released and exchanged between plants. A network of nanosensors can be build up around classical sensor devices already deployed in agriculture fields to monitor these ongoing processes, in addition to other classical physical magnitudes such as humidity or temperature.

1.3 Problems statement

Single transmitter receiver nanonetworks scenarios have been studies in many cases. For example [23] in which it propose the study of reliability estimation in nanonetworks, single-hop nanonetworks which is its communication model just take one transmitter-receiver pair into consideration.

Nonetheless it is inadequate to merely consider single transmitter-receiver pair scenarios. In the computer networks it is a well known that multiple servers receive the requirements from multiple clients and then the servers response to the request that send some specific data such as webpages, voice, videos back to the clients. Also in peer-to-peer networks although without centralized servers, the distributed peers do information transmission and reception simultaneously. So based on these successful application in the classic communication networks we have compelling reasons to believe that in nanonetworks multiple transmitter receivers scenarios are also a important research direction.

In the thesis we study the behavior of multiple transmitter-receiver pairs simultaneously communication in bacterial nanonetworks. Most importantly we focus on

the time delay as the main metrics in the system. We observe the average delivery time, 0.95 quantile delivery time as well as *Cumulative Distribution Function (CDF)* and *probability distribution function (pdf)* of the time distribution of delivery time.

1.4 Thesis structure

The rest of the thesis is organized as follows: Section 2 talks about the theoretical foundation of the thesis work. Markov chain and conjugation process are introduced in detail. Basic concepts of conjugation are presented in here. We define the Markov chain in mathematics language, also we give you some examples of it, in particular we discuss the specific Markov chains which are used in the system briefly.

In Section 3 research method and materials are shown. System model and bacteria movement model are introduced in here. Single/multiple scenarios simulation details are discussed in this section, and all the important equations and illustrations are presented. The problems that we have proposed are addressed at here. Part of the contents are from [9].

The task of Section 4 is to present the results of our simulation and the discussion. In the section we show the diagrams which generated from the data by using matlab, also we discuss the delivery time distribution, mean delivery time, 0.95 quantile metrics in detail. Conclusions are drawn in Section 5.

2. THEORETICAL BACKGROUND

As we mentioned above nanotechnology especially nanonetworks have become one of the most active research topics in the academic realm, unlike the conventional internet linking the computers, laptops, tablets, mobiles via wired or wireless networks, nanonetworks connect nanomachines such that the messages can be transmitted from the transmitter to the receiver.

Our study mainly focuses on communications in nanonetworks, the objective of communications is to send the messages from the transmitter to receiver. A transmitter emits a certain number of bacteria which carry some certain messages, those bacteria randomly move in the experimental environment aiming the receiver. Once one bacterium (we might have many bacteria in the environment but we only care about the first one which reaches the receiver, in other words the transmitting process is done when the first bacterium gets to the destination.) meets with the receiver then we record the time interval from the beginning to the end as one sample of absorption time.

By intuition people should aware that the number of bacteria affect the value of absorption time. Although we can not say the more message carrying bacteria the less absorption time, one thing is clear that the more bacteria will substantially increase the probability that one of them hits the goal. Based on this we try to find some sensible methods to increase the number of message carrying bacteria in our model, on one hand we can make the transmitter emit more than one bacterium and study the absorption time and check whether it has been decreased. On the other hand free bacteria are interspersed in the environment and message carrying bacteria can conjugate with free bacteria such that the number of message carrying bacteria is increased.

In this chapter we first discuss nanoscale communication model, then we show the conjugation process which is one of the critical processes that happens in the implementation. Third, we describe the Markov chain, give its definition and present the Markov model in our system.

2.1 A novel communication paradigm

2.1.1 Communication model

It is straightforward that in nanonetworks we can not connect these nanomachines with cables or fibers like they are used in the internet, and of course the classic wireless networks connection and TCP/IP protocols suite do not naturally suit for nanonetworks as well. So scientists have to revise the communication paradigms in order to make nanonetworks work. Molecular communication is one of the feasible approach for nanoscale communications. Molecular communication is defined as the transmission and reception of information by means of molecules. The different molecular communication techniques can be classified according to the type of molecule propagation in walkway-based, flow-based and diffusion-based communication [17]. In this thesis we mainly discuss the walkway-based molecular communication, it refers to message propagation by using carrier entities such as molecular motors. Also it can be accomplished via the media called chemotaxis. In this thesis when we mention chemotaxis we mainly refer to bacterial chemotaxis.

Bacterial chemotaxis move from the source nanomachines to the destination nanomachines such that the messages are delivered. Generally speaking the movement of a bacterium is made of two different phases: swimming and tumbling. The movement looks like random walk in a manner of relatively straight swimming with alternatively random tumbling which change the moving directions when a bacterium swimming in an uniform environment. Bacteria such as *Escherichia coli* (*E.coli*) are not able to choose the direction in which they swim, also they are incapable of swimming in one single direction for more than a few seconds because of rotation diffusion. In other words, bacteria have amnesia, they do not remember the direction which they are moving to. However, the bacteria are not going to lose themselves at all. Actually bacteria are quite intelligent, they keep sensing the movements to adjust the routes, they can direct their motion to the place where they prefer. If the bacterium senses that it is moving in the correct direction, it will move straightly a little longer time before tumbling. On the contrary if it detects that it is moving in a wrong direction, it will tumble sooner and randomly try one new direction. In fact this kind of sophisticated decision-making process is also a functionality of higher level creatures such as human-beings, it is quite astonishing that such a small bacterium like *E. coli* can possess such complicate intelligence.

In particular our work focuses on messages delivering via flagellated bacteria. Messages can be encoded into DNA strands. The messages carrying DNAs then are injected into bacteria, this procedure is called encapsulation. After encapsulation the bacteria are sent to the propagation channel, the bacteria do Brownian movements

in the channel, essentially they move randomly towards the receiver side. Once the bacteria close to the receiver that the messages are decapsulated and finally decoded. The communication process is illustrated in Fig. 1. We can see that the process parallels its internet counterpart which also pack the data at the source and unwrap it at the sink side layer by layer. However it contains some revisions indeed as we mentioned before and we are going to discuss the communication process in nanonetworks in detail later.

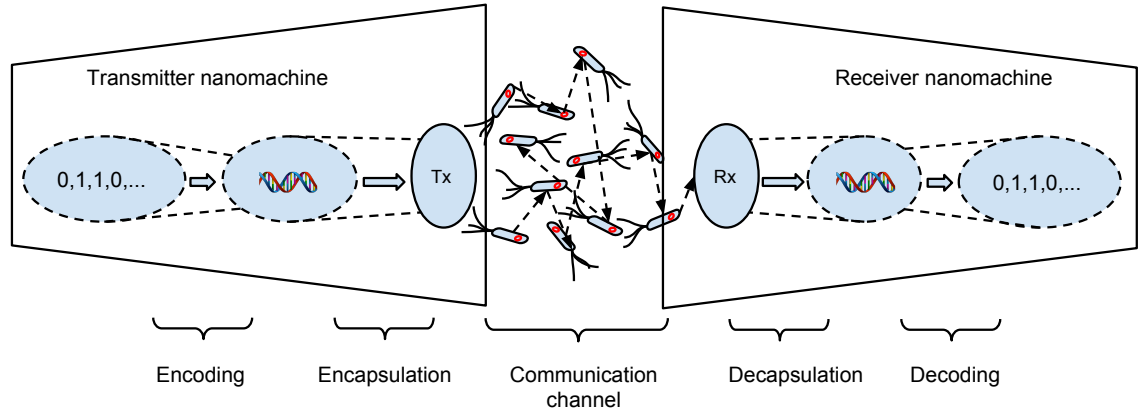


Figure. 1: Bacteria based communication model

We have mentioned previously that in order to make nanonetworks work scientists have revised the classical communication paradigms such that the new paradigm suits for the nanoscale communication. We also have described that in this thesis we focus on flagellated bacteria especially *E.coli* based nanonetworks.

As they are discussed in [20] bacteria have spent several billion years developing skills and efficient machinery, as cilia and flagellum that allow them to convert chemical energy into motion. For instance, *E. coli* has between 4 and 10 flagella, which are moved by rotary motors, placed at the cell membrane, and fuelled by chemical compounds. *E. coli* also has several pili distributed around its outer membrane that give the bacterium the ability to cohere other cells in order to exchange genetic material. This is done by following a cellular process called bacterial conjugation.

The length of *E. coli* is approximately $2\ \mu\text{m}$ and $1\ \mu\text{m}$ in diameter, generally speaking it is a non-harmful bacterium that lives in the human intestine. Its nucleoid contains only one circular DNA molecule and in its cytoplasm there are some smaller DNA sequences arranged in a circular way. These DNA circles are called plasmids which can make the bacteria immune to some antibiotics in the environment. Not only used for protecting the bacteria but also plasmids are used in genetic engineering in order to conduct genetic manipulation experiments.

In the thesis we use *E. coli* as the instance of chemotaxis to send DNA messages to the targeted receivers. First, one particular type of the bacteria which only respond to a specific set of attractants is selected. Second, the DNA messages are injected into the bacteria cytoplasm. Next, the bacteria are emitted to the environment by nanomachines(transmitters), on the other hand the receivers continuously release attractant particles and the emitted bacteria intrinsically drive themselves to the preset receivers follow the attractants. The communication process is achieved through the following steps [22](as it's shown in Fig. 1):

- Encoding and Transmission: In encoding phase the DNA packet is inserted into bacteria's cytoplasm. Different genetic engineering approaches can implement this process, For instance plasmids, bacteriophages or *Bacterial Artificial Chromosomes (BACs)*. As they are well-known and vastly used in biology and pharmacy industry so we discuss them in more detail:
 - Plasmids are circle shaped sequences of DNA, with length between 5.000 and 400.000 base pairs. It needs three steps to encode the DNA packet in the plasmid. First, the plasmid is cleaved in the restriction sites by restriction endonucleases. Second, the DNA packet containing the desired information is added and linked to the plasmid by means of DNA ligase. Finally, the plasmid is inserted inside bacteria's cytoplasm using transformation or electroporation techniques.
 - Bacteriophages are a type of viruses, which are much smaller than bacteria (Bacteriophages range between the 20 and 200 *nm*), and are able to infect bacteria with its genetic material. For instance, Bacteriophage λ vectors have been developed and can be easily cleaved into three pieces, using restriction endonucleases. Two of the pieces contain the essential genes of the phage, but the other one is called filler, and can be discarded and replaced with the target DNA. The bacteriophage with the DNA packet in its genome will infect the bacteria, so the molecular information will be encoded inside the bacteria.
 - BACs are artificial plasmids designed for cloning long segments of DNA. The procedure used to encode the message inside the BAC is the same than for plasmids. However, in this case, the host bacteria must be genetically modified in order to allow the entrance of the long BAC vector through the membrane.

In the earlier implementations, *E. coli* libraries could be created, where each *E. coli* will have different pre-established encoded information, and different DNA packets as well. These bacteria could be stored in the gateway node,

in a kind of warehouse, and each bacterium will be resistant to a specific antibiotic which will allow the selection of the correct bacterium. By applying the antibiotic to a small group of bacteria, the gateway can select and release the desired bacterium, which contains the desired DNA information, to the medium when it is necessary (the other bacteria will die by the effect of the antibiotic). Since *E. coli*, as all bacteria, are able to reproduce, so create a new bacteria with the same genome, new bacteria are constantly created, this ensures that the warehouse will never be empty. It is important to remark that selecting the bacterium by using antibiotics and having pre-established encoding information, will simplify the design of the gateway node in early implementations, however, it will also limit the capacity of the system. Hence, more research is required on how to implement the encoding schemes inside the gateway node.

- **Propagation:** Bacteria have a great number of chemical receptors around its membrane that allow them to sense the environment for the presence of attractant particles and move towards the direction it finds the best living conditions, this process is called chemotaxis. Bacterial chemotaxis is a nature marvel example of signal transduction and it is being widely studied. *E. coli* moves in series of runs and tumbles. In each run, the flagella motors spin counterclockwise, and the bacterium swims approximately in a straight line. Whereas, a tumble is a small period of time where the bacterium moves erratically in the same place due to one or more filaments are spinning clockwise. During a running period, the bacterium senses the amount of nutrients (sugars, amino acids, dipeptides) in the environment several times, using cell membrane's chemoreceptors. Comparing the obtained results, the bacterium is able to decide whether the nutrient concentration is increasing or decreasing. If the concentration is increasing, the running time is longer. This bias in the running time enables cells to find the places where the environment is better.
- **Reception and Decoding:** The outer part of the gateway may be a cellular membrane. Hence, the carrier bacterium sees the gateway node as a receiver cell and will follow its natural instincts and will pass the plasmid to the gateway node. This process is called Bacterial Conjugation defined as the exchange of plasmids among bacteria cells. In order to carry out the exchange of genetic material, direct contact is required, which is achieved by means of the bacterial appendage called pilus. This contact makes both membranes to fuse together, in a kind of bridge by which the donor bacterium transfers a single strand of the plasmid. Once the plasmid is in the receiver's gateway, the DNA packet must be extracted from the plasmid. This is done by restriction endonucleases

enzymes that cleave the plasmid in restriction sites. DNA computers are able to separate different DNA strands by lengths, this allows the gateway node to recover the DNA packet among the solution containing both the cleaved plasmid and the DNA packet. Then, the gateway is able to process the DNA packet as required.

2.1.2 Expected behaviors

In molecular nanonetworks we anticipate the following behaviors which are distinct from the corresponding behaviors in the internet [25].

- Chemical signals are encoded/decoded: in nanoscale communication chemical signals are encoded and sent from the transmitter nanomachines to the receiver nanomachines. For instance, a message can be encoded into DNA sequence and then be propagated, this is the typical scenario that is used in our system.
- Slow speed and large delay: we've mentioned that bacteria swim from the transmitter to the receiver nanomachine with random movements such as Brownian motion or random walk. The moving speed is very low compare to the transmission speed of the internet. Consequently the time delay in nanonetworks is much higher than in the classical networks. More importantly in this thesis work we mainly focus on the delivery time performance in our system.
- Biocompatibility: transmitters and receivers that used in nanonetworks have the same attributes as the entities of biological systems, they also share common communication schemes. As a result the application of nanonetworks are naturally compatible with the biological system such as human body.
- Energy efficiency: since nanoscale communications inherently follow some of the mechanisms of biological system, their energy efficiency is high. For instance, the implant human body nanomachines can generate energy (e.g. via glucose) so that they can empower themselves.

2.2 Conjugation

One more important concept we are going to introduce is conjugation. In short conjugation is a replication process. During the transmission process there are free bacteria also moving in the propagation channel together with message bearing bacteria, those bacteria which are taking messages can copy the messages to the free ones through conjugation process, after conjugation free bacteria become message carrying bacteria. The increasing number of message carrying bacteria aggrandizes

the probability of the bacteria reaching the receiver which means the deliver time is likely decreased. Conjugation process is also a stochastic process, each time when two bacteria meet there exists a conjugation probability. Conjugation either succeed or fail depends on the probability.

There are two major properties which makes the flagellated bacteria a useful concept for nanoscale communications: (i) as any other bacteria or cell, it has the ability to store DNA-encoded data in chromosomes or plasmids, (ii) bacteria are able to pick up swimming DNA particles through the process of transformation [1]. Finally, the inherent ability to swim in the environment may potentially enable delivery of encoded messages to a communicating party, e.g. a receiver nanomachine.

Among various bacteria of these two properties *E.coli* is the most studied one, the studies of *E. coli* can be traced back into the 1880s. In 1885, the German-Austrian pediatrician Theodor Escherich discovered this organism in the feces of healthy individuals and called it *Bacterium coli commune* because it is found in the colon and early classifications of prokaryotes placed these in a handful of genera based on their shape and motility (at that time Ernst Haeckel's classification of bacteria in the kingdom Monera was in place). *Bacterium coli* was the type species of the now invalid genus *Bacterium* when it was revealed that the former type species ("*Bacterium triloculare*") was missing. Following a revision of *Bacterium*, it was reclassified as *Bacillus coli* by Migula in 1895 and later reclassified in the newly created genus *Escherichia*, named after its original discoverer. Also *E.coli* naturally exists in human body which makes the study of it more sensible and applicable for practical meaning. Most *E. coli* are not harmful, the harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2 , and preventing colonization of the intestine with pathogenic bacteria [18]. Also in our system plasmid is the main entity for achieving conjugation, so next we are going to introduce plasmid.

2.2.1 Plasmid

A plasmid is a small, circular, double-stranded DNA molecule that is distinct from a cell's chromosomal DNA. Plasmids naturally exist in bacterial cells, and they also occur in some eukaryotes. Often, the genes carried in plasmids provide bacteria with genetic advantages, such as antibiotic resistance. Plasmids have a wide range of lengths, from roughly one thousand DNA base pairs to hundreds of thousands of base pairs. When a bacterium divides, all of the plasmids contained within the cell are copied such that each daughter cell receives a copy of each plasmid. Bacteria can also transfer plasmids to one another through the conjugation process [2].

Attributes of plasmid

Plasmids have several important properties which are quite essential for us [3], our experiments are based on plasmids because of the following properties:

- Easy to work with:

Morden cloning technology assures that plasmids containing the genetic information that people are interested in can be easily created and manipulated. Obviously it is a practical factor that is available for nanonetworks because of it's accessibility.

- Self-replicating:

Plasmids intrinsically replicate themselves such that more and more plasmids with the same information are created, the bigger amount of plasmids the easier for conjugation process.

- Stability:

Plasmids are stable long-term either as purified DNA or within bacteria. So the encoded messages will not get lost by accident.

- Diversity:

Plasmids can drive gene expression in a wide variety of organisms, including plants, worms, mice and even cultured human cells. Although plasmids are commonly used to understand gene function, they can also be used to investigate promoters, small *Ribonucleic acid (RNA)*, or other genetic elements.

2.2.2 Conjugation process

Conjugation is the process which one bacterium transfers genetic material to another through direct contact. During conjugation, one bacterium serves as the source of the genetic material, and the other serves as the destination. The source bacterium carries a DNA sequence called the fertility factor, or F-factor. The F-factor allows the donor to produce a thin, tubelike structure called a pilus(in plural: pili), which the source uses to contact the destination [4]. When physical contact has been established via pilus, plasmids with certain genetic information are copied from the source to the destination via the pilus. A basic conjugation process is illustrated in Fig. 2. In our system conjugation process makes the the information that we are interested in copied, furthermore other information which emitted by other transmitters are also able to copied to free bacteria through conjugation(multiple transmitter-receiver pairs case), such case makes the system more complicate, those bacteria which carrying our messages possess relatively smaller amount of free bacteria compare to

single transmitter-receiver scenarios. We intentionally introduce the free bacteria competing process to the system in order to study how can the interference rework the system behavior. Because multiple transmitter-receiver pairs scenarios have not been widely studied before, we believe they are worth researching.

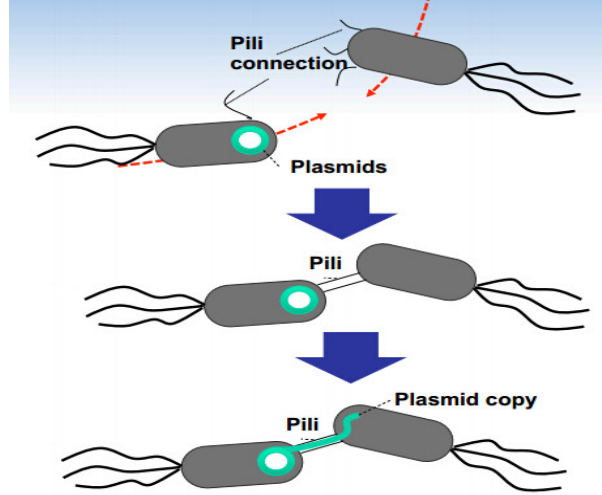


Figure. 2: Conjugation process [5]

2.3 Markov chain

Markov chain is a mathematical tool used to model various stochastic processes through states and transitions. It is also considered as the random version of finite automaton, each state can either move to the other states or stay in the same state with certain probabilities. Although theoretically the state space of Markov chain can be infinite in the real world usually it is finite or countably infinite. A Markov chain must have the memoryless property, we are going to present it in the *Memoryless property* section.

2.3.1 Introduction to Markov chain

In this work we simulate the single/multiple pairs scenarios with Markov chain model, the code is in matlab. The theoretical foundation of Markov chain is memoryless distribution, in specific exponential distribution and geometric distribution. These are the only two memoryless distributions, naturally we know that there are some internal relation between exponential distribution and geometric distribution, in fact geometric distribution can be considered as the discrete analogue of exponential distribution which describes independent Bernoulli trials. The property of memoryless distribution is that the next state on the chain only depends on the current state which has not any connection with the previous states. This essential property inherently suits for Markov chain, particularly we assume the delivery

time distribution follows exponential distribution. Furthermore we concern the absorption time of the chain, the absorption time is an equivalent concept of deliver time. In our model we have a starting state: S , one accepting state: A , the rest of states are inter-states between S and A . In fact the absorption time is calculated by adding all the elapsed time from S to A . Due to the randomness of the communication process each time we get different trajectory and different absorption time. We conduct the same experiment for a large amount of time and collect all the absorption time to get statistical results, theoretically the more measurements the more accurate results we can get, while in practice we can not increased the numbers of measurements without limitation since each measurement takes some time. After data collection we pass all the data to diagrams plotting function, after execution of the code we will get CDF, mean deliver time, 0.95 quantile diagrams. In total we are able to generate more than one hundred diagrams which provide us a solid ground for our research work.

We implement single transmitter-receiver pair and multiple transmitter-receiver pairs scenarios separately:

- In single pair cases we vary the parameters: size of the compartment, the number of free bacteria and emitted bacteria. By changing these parameters we try to find out the how these variables effect the distributions of absorption time.
- In multiple scenarios we fix the compartment size and the number of free/emitted bacteria are still variable, in this case we are going to figure out the interference of other transmitter-receiver pairs and how the absorption time distributions are correlated to these parameters.

In this section we introduce the theoretical matters of Markov chain, first we will describe Markov chain from the state space's point of view, next we give the formal definition of it, finally we discuss memoryless property.

Description

We describe a Markov chain as follows: We have a set of states, $S = \{s_1, s_2, \dots, s_r\}$. The process starts in one of these states and moves successively from one state to another. Each move is called a step. If the chain is currently in state s_i , then it moves to state s_j at the next step with a probability denoted by p_{ij} , and this probability does not depend upon which states the chain was in before the current state. The probabilities p_{ij} are called transition probabilities. The process can remain in the state it is in, and this occurs with probability p_{ii} [6].

Next we give a straightforward example of Markov chain. In order to show it in a accessible way we have depicted a state transition diagram as in Fig. 3:

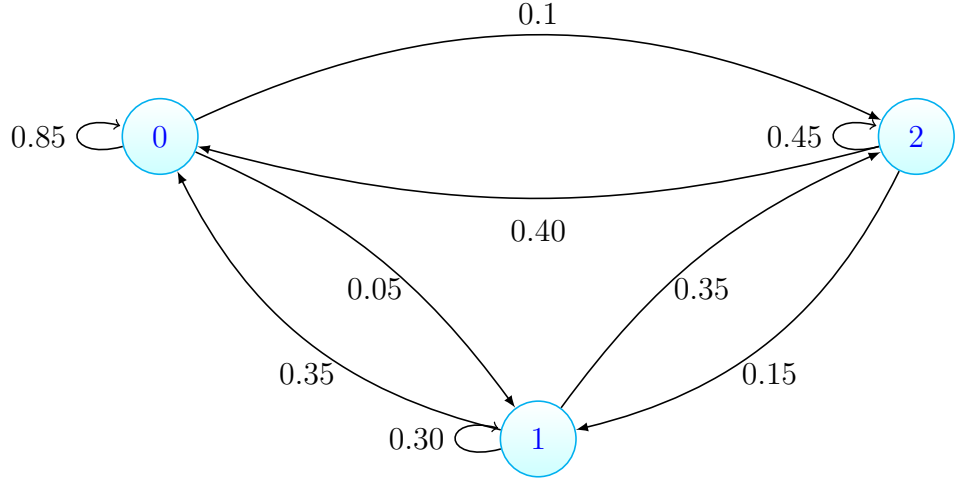


Figure. 3: Markov chain example.

Definition

A Markov chain is a sequence of random variables $\mathbf{X}_1, \mathbf{X}_2, \mathbf{X}_n, \dots$ with the Markov property, namely that, given the present state, the future and past states are independent. Formally,

$$P_r(\mathbf{X}_{n+1} = x | \mathbf{X}_1 = x_1, \mathbf{X}_2 = x_2, \dots, \mathbf{X}_n = x_n) = P_r(\mathbf{X}_{n+1} = x | \mathbf{X}_n = x_n), \quad (2.1)$$

if both conditional probabilities are well defined, for instance if $P_r(\mathbf{X}_1 = x_1, \mathbf{X}_2 = x_2, \dots, \mathbf{X}_n = x_n) > 0$. [7]

The state transitions can also be presented as transition matrix, consider the following transition matrix:

$$P = \begin{bmatrix} 0.85 & 0.05 & 0.1 \\ 0.35 & 0.30 & 0.35 \\ 0.40 & 0.15 & 0.45 \end{bmatrix}.$$

People may notice that it is the transition matrix of the state diagram shown above. We can easily see that from each state it has three possible transitions with some certain transition probabilities. In here the sum of the probabilities from one single state is always 1 which means each state can have a successor for sure. Meanwhile one state does not necessarily have a succeeded state, for instance in our system we have an absorption state or accepting state, after arriving the absorption state it does not go to anywhere further. More information about absorption state is shown in 2.3.2.

Memoryless property

Memoryless property which means that the probability distribution of the next state depends only on the current state, it has nothing to do with the rest of other states. In (2.1) we can see that the conditional probability of the next state given all the previous states $P_r(\mathbf{X}_{n+1} = x | \mathbf{X}_1 = x_1, \mathbf{X}_2 = x_2, \dots, \mathbf{X}_n = x_n)$ equals to the conditional probability given only current state $P_r(\mathbf{X}_{n+1} = x | \mathbf{X}_n = x_n)$. This property is key factor to Markov chain, without it Markov chain would become infeasible.

Two kinds of random distribution have Markov property which are exponential distribution and geometric distribution. In the implementation we presume that the distribution follows exponential distribution. Next we briefly explain the concept of exponential distribution.

The probability distribution function of exponential distribution is:

$$f(x; \lambda) = \begin{cases} e^{-\lambda x} & x \geq 0, \\ 0 & x < 0. \end{cases} \quad (2.2)$$

Next we prove the memorylessness of exponential distribution [8]:

$$P(X > t) = \int_t^{\infty} \lambda e^{-\lambda x} dx = \lambda \frac{-e^{-\lambda x}}{\lambda} \Big|_t^{\infty} = e^{-\lambda t} \quad (2.3)$$

$$\begin{aligned} P(X > s + t | X > s) &= \frac{P(X > s + t, X > s)}{P(X > s)} \\ &= \frac{P(X > s + t)}{P(X > s)} \\ &= \frac{e^{-\lambda(s+t)}}{e^{-\lambda s}} \\ &= e^{-\lambda t} \\ &= P(X > t) \end{aligned} \quad (2.4)$$

□

2.3.2 Implementation of Markov chain

As we mentioned in our simulation we have two basic scenarios: (i) single transmitter-receiver pair, (ii) multiple transmitter-receiver pairs. In order to represent both cases we design two types of Markov chain for single transmitter-receiver pair cases and multiple transmitter-receiver pairs cases respectively. The main difference between single cases and multiple cases is that in the multiple ones we have introduced interference to the chain, interference is meant by those bacteria which are taking

other messages can also conjugate with free bacteria. Vividly we call the single case Markov chain a 2-dimensional chain because each state except the absorption state has two possible transition to go, similarly the multiple transmitter-receiver case Markov chain is called a 3-dimensional one since every state has three possible ways to move besides the absorption state.

We have a starting state in the chain meaning that at this specific time point transmitter(s) has just emitted the message carrying bacteria and we have an absorption state which represents the first bacterium carrying our message reaches the receiver. The absorption state is like a black hole that anything goes inside it can never go out. In the transition matrix absorption state can be denoted by every possible transition has 0 probability.

Due to the randomness of the delivering process the number of inter-transition states are random, that is, we can not depict a certain Markov chain like which is shown in Fig. 3 saying that this is the model of our system. Each round of simulation we have different number of inter-transition states. Another misunderstanding which people commonly maintain is that the longer the Markov chain the longer the absorption time, it seems correct by intuition. However, because of randomness we can not consider that the absorption time is proportional to the length of the Markov chain.

Next we give a counter-example which shows that a longer Markov chain does not necessarily have a longer absorption time, consider two Markov chain instances of a certain scenario, *Markov Chain Instance (MCI)* A and MCI B, in other words they are two various measurements of the scenario. In MCI A there are states denoted by $S_{MCIA} = \{sa_1, sa_2, \dots, sa_i\}$, MCI B contains states denoted by $S_{MCIB} = \{sb_1, sb_2, \dots, sb_j\}$, $i < j$ which means A is shorter than B. At the mean time MCI A has the transition time for each pair of states denoted by $T_{MCIA} = \{ta_1, ta_2, \dots, ta_{i-1}\}$, likewise we have the transition time of MCI B $T_{MCIB} = \{tb_1, tb_2, \dots, tb_{j-1}\}$, apparently each transition time is bigger than 0. We calculate the absorption time of the two Markov chains by the following equations:

$$T_{absorb_A} = \sum_{m=1}^{i-1} ta_m \quad (2.5)$$

$$T_{absorb_B} = \sum_{n=1}^{j-1} tb_n \quad (2.6)$$

it is fair to assume that $\sum_{m=1}^{i-2} ta_m = \sum_{n=1}^{j-1} tb_n = T_{absorb_B}$, so $T_{absorb_A} = T_{absorb_B} + ta_{i-1}$, since $ta_{i-1} > 0$ we get $T_{absorb_A} > T_{absorb_B}$.

2.4 Summary of theoretical background

Basic data transmission in nanonetworks can be achieved by single transmitter-receiver nanomachine pair, meaning that there is no competition when message bearing bacteria want to conjugate with free bacteria, it is also the commonly studied transmission model in the research area. However in our work we intend to study the multiple transmitter-receiver pairs scenarios, in these scenarios interference is introduced to the system, different transmitters emit various messages, in which we only concern the message that our specific source emitted, other messages are noise in the system. Intuitively we know that noise deteriorate the effect of communications, however the way that noise interrupt the communications in nanoscale systems is different from the one in conventional communications. We know that we do not have the classical noise. The issue is those emitted bacteria other than ours can also conjugate with free bacteria, since the number of free bacteria is a constant, once a bacterium which taking other messages is conjugated with a free bacteria, the total number of free bacteria decreased, from our message sending point of view, those bacteria carrying our messages have relatively lower amount of free bacteria for conjugation, obviously the efficiency of communications is interfered, in other words this arouse the noise in the system. That's why multiple transmitter-receiver scenarios are more complicate than the single ones. Also it's the reason that we are interested in multiple scenarios, in this thesis we attempt to analysis their behaviors in transmission and compare their performance with the single case scenarios.

Finally combining the data and the diagrams that we acquired, based on the data we analysis the results, as a outcome this thesis work gives us some conclusions regarding simultaneous communications in bacterial nanonetworks, also it is the basis of our paper publication.

3. THE MODELING FRAMEWORK

In order to address the problems that we have proposed we need to build up a simulation environment such that one transmitter release a certain number of message carrying bacteria, those bacteria inside the experimental container move randomly toward the receiver. During the movement they can conjugate with free bacteria, the procedure of conjugation is a stochastic process. More importantly we construct the environment which exists multiple transmitter and receivers pairs, each individual transmitter emits its particular message bearing bacteria, certain bacteria random move aiming to their own specific receiver. Although we consider the scenario the multiple transmitter-receiver pairs system as a whole, in this case we merely care about one pair of them especially since those bacteria which are emitted from it carry the messages that we are interested in.

We implement both cases respectively in matlab and record the delivery time of each transmission process as raw data. As to make our simulations more representative, during the implementation the parameters such as number of emitted bacteria, number of free bacteria, size of the compartment, etc. are able to be modified. In addition, we analysis the raw data, and find out the distribution of the delivery time, mean delivery time and 0.95 quantile of the delivery time as well. These three metrics give us the statistic foundation of our work that our conclusions are based on them.

We put a bit more concentration on multiple transmitter-receiver pairs scenarios because of the following three reasons: (i) multiple cases are rarely studied previously. (ii) multiple cases are important, sensible and more practical from the applications' point of view. (iii) conjugation processes in multiple transmitter-receiver pairs scenarios are more complex.

In this chapter we first present the system model in detail, various of parameters are introduced at here. Second, we describe the bacteria movement model including movement pattern, metrics, first- and inter-passage times, single and multiple transmitter-receiver models. Finally we list some code in matlab which improve the efficiency of our implementation.

3.1 The system model

3.1.1 Principles of bacterial communications

As it was shown in Fig. 1 the process of communication in bacterial nanonetworks can be defined as (i)encoding of data, (ii)encapsulation, (iii)channel propagation, (iv)decapsulation, and (v)decoding.

The first two and last phases are mirrored ones performed at the transmitter and receiver sides. First, message is encoded into the DNA strand at the transmitter nanomachine. At the second stage the information in terms of DNA strands is picked up by bacteria via the so-called transformation process. Depending on whether a single or multiple bacteria will be emitted by a nanomachine it could also be replicated to enable multiple bacteria carrying the same information. Replication could be achieved via supplying food and waiting for a certain amount of time for bacteria to replicate. Alternatively, a bacteria could be injected in a compartment having other bacteria inside. In this case the information is replicated to a number of bacteria using the conjugation process explained below. At the receiver these operations are performed in reverse order, that is, we first decapsulate the information and then decode it.

In this study we are interested in channel characteristics, that is, actual delivery of information via bacteria movement in a certain compartment. During this phase, bacteria with DNA-encoded messages are released from the source nanomachine, propagate through the medium and, finally, reach the destination nanomachine, where it captured. Taking into account the speed of movement of flagellated bacteria and its random patterns the time till bacterium reaches the receiver nanomachine could be extremely large. Three mechanisms improving performance of information transmission have been proposed in the past:

- chemioattraction;
- multiplication;
- conjugation.

Chemioattraction is the process of emitting a certain chemicals to the environment that affect the behavior of the bacteria moving patterns. For example, putting some sugar in the environment at a certain separation distance from a bacterium could cause bacteria to move following the gradient of concentration towards the attractant point. Mathematically, the unbiased random walk become biased with drift towards the maximum concentration point. The question of suitability of chemioattraction in bacterial nanonetwork is still open. Indeed, for efficient use of this phenomenon the receiver should know when the source is going to transmit which rarely

happens in practice. Another issue is removal of chemioattractants from the environment once transmission is over. Finally, multi-transmitter-receiver communications could be prohibited in the same compartment.

The multiplication is a primitive mechanism referring to increasing the number of bacteria carrying the information or the number of receivers for a given source. Assuming independence of individual bacteria movements and recalling basic facts from the random walk theory we could expect exponential increase in delay performance increase both parameters.

Finally, conjugation refers to exchange of genetic information between bacteria. When two bacteria are close to each other and have enough of resources in terms of internal energy they may remain fixed for a certain amount of time and exchanging plasmids containing DNA. This feature is critical for survivability of bacteria. That is, if there is a certain amount of the so-called free bacteria in the environment they all can be used for information delivery via conjugation process. The model proposed next captures both multiplication and conjugation for improving delivery performance of bacterial nanonetworks [9].

In this thesis both of two scenarios will be discussed. Multiple transmitter-receiver pairs scenario are considered as a valuable extension of the single case. We study more about multiples scenarios in order to develop the bacterial nanonetworks research into a new level. In this work we develop the model which is inherently analytical such that we are able to evaluate qualitative effect of various input parameters and reveal hidden trade-offs between them. Markov chain is used as the analytical model of the system for both scenarios.

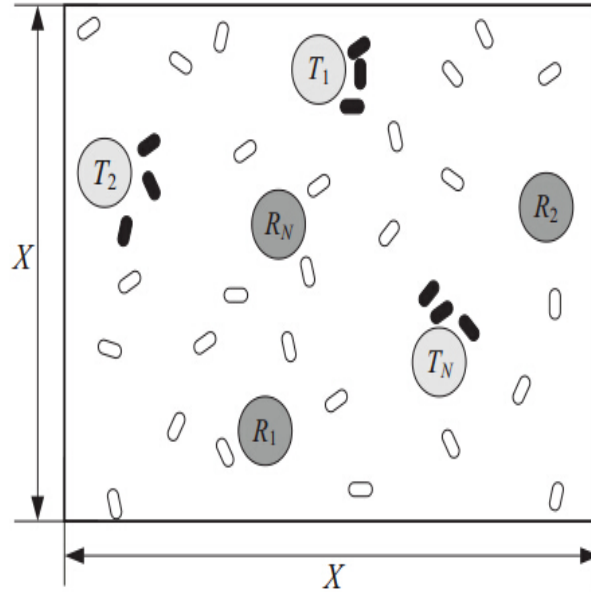


Figure. 4: An illsutration of the system model.

3.1.2 System model

We consider a squared $X \times X$ environment with N transmitter-receiver pairs as shown in Fig. 4. Positions of transmitters and receivers are uniformly distributed over X^2 . Each transmitter emits B_E bacteria carrying identical information. The length of all messages is identical and equal to L_M informational units measured in DNA base pairs. These messages are inserted into plasmids that are further injected into bacteria at the transmitter. In order to transmit informations each transmitter releases B_E bacteria containing the whole message into the environment. The radius of a single bacteria is $r_B \ll X$. We also consider the case of $N_R \geq 1$ receivers for a single transmitter.

Parameter	Meaning
X	Side of a compartment
N	Number of transmitter-receiver pairs
B_E	Number of emitted identical bacteria from a source
B_F	Number of "free" bacteria in the environment
N_R	Number of receivers for a single transmitter
r_R	The radius of receiver
R_B	Sensitivity radius of a bacteria
p_C	Probability of conjugation
c	Conjugation rate (pairs per second)
L_M	The length of a message
μ	Environment shaking process
v	Bacterium swimming speed
τ	Bacterium mean inter-tumble time
α	Bacterium tumbling angle

Table 3.1: Notation used in this thesis.

There are B_F bacteria uniformly distributed over X^2 . We call these bacteria "empty" or "free" as initially they do not contain any message. These bacteria help to deliver the message to the receiving nanomachines through the process called conjugation. The sensitivity radius of a bacteria $r_B (r_B \ll X)$ is an area of circular shape around a bacterium. Once two bacteria are within the reach of each other the conjugation process starts with probability p_C . During the conjugation the original message is copied to another bacterium at rate C base pairs per second. The system as a whole is subject to environmental shakes occurring according to the Poisson process with mean μ . Once the system is shaken the conjugation process stops abruptly and two bacteria continue to move randomly. Once the receiver nanomachine is reached the message is considered to be delivered. The radius of all receivers is r_R . We are interested in delay-related metrics of the information

delivery process including moments and distributions, especially we concern about pdf, CDF, mean delay, and 0.95 quantile.

3.2 Bacteria movement model

3.2.1 Empirical movement pattern

According to empirical model in [11], in absence of external stimulus (i.e. chemioattraction) a bacterium moves interchanging straight runs and short tumbles. That is, it first chooses a direction arbitrarily in between 0 and 2π by swirling around and then moves along the straight line for exponentially distributed time τ at constant speed v . The latter results in exponentially distributed distances between two stopping points. The above-mentioned empirical model is heavily studied in various applied fields including communications in context of ad-hoc networks, where it is called a *random direction model (RDM)* (see e.g. [10] for properties). In random walk theory it is referred to as Pearson-Rayleigh random walk.

3.2.2 Performance metrics

In classic electromagnetic communications systems one of the most important performance metrics is the mean delay between of message transfer between two communicating entities. Usually, the propagation time component of the message delay is constant for a given medium. Using freely-swimming bacteria as an information carrier it is easy to see that the propagation delay is a random variable. Furthermore, the situation is complicated by the dimension of interest. Observe that the bacterium mobility process is a special case of a Markov process. In order to show it let the stochastic process $\{S_n, n = 0, 1, \dots\}$ denote the distance from the origin (or, generally, from any point in \mathbb{R}^2) at the tumble n . The time evolution can be described as

$$S_{n+1} = \sqrt{(S_n + l_{n+1} \cos \phi_{n+1})^2 + l_n^2 \sin^2 \phi_{n+1}} \quad (3.1)$$

where $\{l_n, n = 0, 1, \dots\}$ is the sequence of independent identically distributed RVs with exponential distribution and $\{\phi_n, n = 0, 1, \dots\}$ is the sequence of iid RVs with uniform distribution. It is easy to show that this process is Markov in nature with all the states being recurrent null. The latter implies that, the random walker returns to the origin (or, alternatively, visits any region of space) with probability one but the mean return time (recurrence time or propagation delay for our model) is infinite. Recall, that for \mathbb{R} all the states are recurrent positive implying that the mean recurrence time is finite. Another case of interest is \mathbb{R}^3 , where the random walk becomes transient implying that not only the mean recurrence time is infinite but the process returns to the initial state with probability less than one. The latter

implies that a certain point in space may never be visited in three-dimensions.

To understand the reason for this behavior one may consider a motion of a Brownian particle in one-dimension with two absorbing boundaries. Brownian motion serves as a special limiting case for many unbiased random walks including the Pearson-Rayleigh walk. Recalling the results for Brownian motion the formal structure of first passage time (FPT), a common term used to refer to recurrence time, distribution is given by a mixture of power law and exponential distributions in the following form

$$f_T(t) \sim t^{-\alpha} e^{-\frac{t}{\beta}} \quad (3.2)$$

where coefficients α and β depends on the dimension of the space and "volume" an area (area in \mathbb{R}^2) and can be related to the diffusion constant of a Brownian motion. When boundaries of an area, where a particle is allowed to move, are getting wider the exponential term disappears and the behavior is fully determined by the power-law behavior. Thus, for open two- and three-dimensions the mean FPT is infinite. For a closed region, $V \subset \mathbb{R}^2$, all states of the Markov process are recurrent positive implying that the chain not only returns to the origin infinitely often but the mean time return time is finite. The general form of the FPT is still given by (3.2) with coefficients α and β depending on the "volume" of V , movement speed, etc.

By definition, the FPT distribution gives us the time of a first contact between bacterium and the receiver nanomachine. As a result, it provides the delay performance of a communications link. Further, using the quantiles of FPT distribution one can get the metric assessing link reliability, i.e. the amount of time it takes to deliver a message for a communicating entity with $x\%$ confidence. Finally, assuming independence of bacteria movement we can provide these metrics for multiple emitted bacteria. Thus, we see that for our system the main performance metrics can be obtained using FPT distribution of a single bacterium.

3.2.3 First- and inter-passage times

The important metrics required to parameterize the model introduced below are *first-passage time (FPT)* and *inter-passage times (IPT)* between two bacteria or a bacterium and a receiver nanomachine. In what follows, we concentrate on FPT and IPT between two bacteria. Similar argument applies for FPT/IPT of a bacteria and the receiver nanomachine. We define the first-passage time as the time passing between a randomly chosen instant of time and the time when two bacteria meet. The inter-passage time is the time between two successive meetings. There are no exact results for FPT metrics available even for a essential Pearson-Rayleigh random walk. Nevertheless, there are a number of approximations that can be used to capture FPT and IPT distributions. We will use one of them in our study.

Let $f(x; y)$ be the distribution of the bacteria location in steady-state. It has been shown in [12] that this distribution for RDM is uniform over X^2 , i.e. $f(x; y) = \frac{1}{X^2}$. Furthermore, following our assumptions both the transmitter nanomachine and all other "free" bacteria are both uniformly distributed over X^2 . Assuming independence of movement patterns, $r_B \ll X$, some speed V , and independence of location of an object at t from its location at $t + \Delta$ for some small Δ it has been shown in [12] Ch. 4 that the FPT approximately follows exponential distribution with rate

$$\lambda_B \approx 2r_B E[v^*] \int_0^X \int_0^X f^2(x, y) dx dy = \frac{2r_B E[v^*]}{X^2}, \quad (3.3)$$

where $E[v^*]$ is the average relative speed between two objects.

When the speed of objects is constant, coincides and equals to v we have

$$E[v^*] = \frac{4v}{\pi}, \quad (3.4)$$

leading to

$$\lambda_B \approx \frac{8r_B v}{\pi X^2}, \quad (3.5)$$

implying that the expected time to meet is $E[T_{FPT}] = \frac{\pi X^2}{8r_B v}$.

Consider now the FPT between a bacteria and the receiving nanomachine. When one object is not moving while the other is moving at constant speed v the equation (3.3) reduces to

$$\lambda_R \approx 2r_B v \int_0^X \int_0^X f^2(x, y) dx dy = \frac{2r_B v}{X^2} \quad (3.6)$$

The above-mentioned results are only approximately valid. Note that the expression for the mean FPT has been independently obtained in [13] under the same set of assumptions but using slightly different approach. One crucial assumption for this result to hold is that the objects move around fast enough. That is, the position of the bacterium at some time $t + \Delta$ is independent of its position at the time instant t for some small t . Recall that the bacteria moves at the constant speed $v = 20$ mcm/s. for exponential time with mean 3.5s leading to the average run length of 70mcm. Thus, the requirement of moving fast enough in our case implies that the dimension of the area X should not be significantly greater than few times of 70mcm. Quantitative bounds on the dimension of the area are scarce. First of all, the FPT distribution in \mathbb{R}^1 for RDM has been studied by Sparre-Andersen in [14], where he demonstrated that the FPT between a moving and stationary object follows power-law. Still empirical results demonstrated by Groenevelt in [12] and Spyropoulos et al. in [13] for RDM in \mathbb{R}^2 shows otherwise.

So far we discussed FPT only. Another important metric we are interested in is IPT. To the best of authors' knowledge there are no analytical results on IPT for

RDM in \mathbb{R}^2 . Still under the set of the above-mentioned assumptions both Groenevelt in [12] and Spyropoulos et al. in [13] demonstrated that the IPT distribution is also exponential and coincides with FPT distribution. Inspired by the study of Groenevelt the question of the form of IPT distribution has been empirically investigated by Zheng [16] [15] who demonstrated that the IPT distribution has a complex behavior consisting of a mixture of exponential and power-law behavior. In particular, if the mean run length of RDM is at least half of the dimension on the area (in our case $X < 140\text{mcm}$) then the distribution is exponential. When X increases there appears to be a turning point up to which the distribution exhibits the power-law behavior while the tail is still exponential. When the dimension of the area increases this point moves to the right direction eventually converging to power-law function for very large X . However, for our purposes the exponential assumption seems reasonable as it allows to get the so called first order approximation for FPT and greatly simplifies further modeling.

Summarizing, we see that the FPT and IPT between two bacteria are exponentially distributed with the same parameter $\lambda_B = 8r_b v / \pi X^2$. The former is due to assumption of uniform distribution of the measuring nanomachine and empty bacteria in X^2 . FPT and IPT between a bacteria and the receiver nanomachine is also exponentially distributed with the same parameter $\lambda_R = 2r_B v / X^2$. Note that it is straightforward to take into account the movement of receiver nanomachines.

3.2.4 Single transmitter-receiver model

Assume that at time $t = 0$ the transmitter nanomachine emits a bacteria having a message of length L_M . Recalling the results of the previous subsection we see that this bacterium hits the receiver nanomachine with rate $N_R \lambda_R$ or engaged in the conjugation process with one of the free bacterium with rate $N_B \lambda_B$. If the former happens then the process ends, that is, the information is delivered to the receiver. If the latter happens then the conjugation process starts with probability p_C . Recall that the meeting process with a single empty bacterium is Poisson in nature with parameter λ_B . Thus, the process of meetings with N_B bacteria is again Poisson with rate $\lambda_B N_B$. Since there is non-zero probability, $1 - p_C$, that two bacteria that come in contact with each other do not start the conjugation process the process of conjugation is Poisson with rate $p_C \lambda_B N_B$.

The conjugation process, when started, may abruptly end due to external shakes the system is subject to. These "shakes" happen according to the Poisson process with intensity μ . Once the system is shaken all ongoing conjugations in the system are aborted. We are interested in only those conjugations that are completely finished, i.e. the whole message is copied. Recalling that the length of the message is L informational units while the copy rate is C units per second while $(1 - e^{-u\Delta t})$ is the

probability of having no events from the Poisson process with intensity in the time interval of length Δt , the probability of successful completion of the conjugation is

$$p_C = (1 - e^{-\mu T}), T = \frac{L_M}{C} \quad (3.7)$$

where T is the time required to successfully copy the whole message.

The above-mentioned discussion implies that the process of information delivery to the receiver nanomachine can be modeled using the continuous time absorbing Markov chain model, $\{S(t), t \leq 0\}$. The state-space is $S(t) \in \{1, 2, \dots, N_B + 1\}$, where states $\{1, 2, \dots, N_B\}$ are transient ones modeling the number of bacteria having the whole message by time $t, t > 0$ while state $N_B + 1$ is absorbing one. The state transition diagram of such model is illustrated in Fig. 5, where the state $N_B + 1$ is denoted as A (absorbing state). From any state i there are only two possible transitions, to the state $i + 1$ and to the absorbing state $N_B + 1$. The rates out of a certain state i are given by

$$\lambda_{i,i+1} = ip\lambda_B(N_B - i), \lambda_{i,N_B+1} = i\lambda_T N_T \quad (3.8)$$

where the parameter p is responsible for taking into account the effects of the conjugation process. The choice of this parameter is extremely important for modeling purposes, depends on other system parameters such as dimension of the compartment X and the bacteria density. Neglecting the effect of conjugation delays (time required to copy the message) on the delay performance of the information delivery system we may set

$$p = p_C(1 - e^{-\mu T}) \quad (3.9)$$

We will discuss how to take into account the effect of conjugation delays in the next subsection. It is also important to mention that applying (3.9) the original Poisson process of meetings between bacteria is probabilistically thinned with probability p and due to the Raikov thinning theorem we get again the Poisson process describing the process of successive conjugation.

The structure of the chain allows for rather straightforward analysis for laplace transform of the delay. Let T be the message delivery delay and $f_T(t)$ be its probability density function. We are interested in *Laplace-Stieltjers transform (LST)* of $f_T(t)$ defined as

$$L_T(\nu) = E[e^{-\nu T}] = \int_0^\infty e^{-\nu t} f(t) dt \quad (3.10)$$

Using the law of total probability we write (3.10) as

$$E[e^{-\nu T}] = \sum_{i=1}^{N_B} E[e^{-\nu T} | S(t) = i] P_r\{S(t) = i\} \quad (3.11)$$

where $S(t)$ is the number of bacteria having the message prior to absorption.

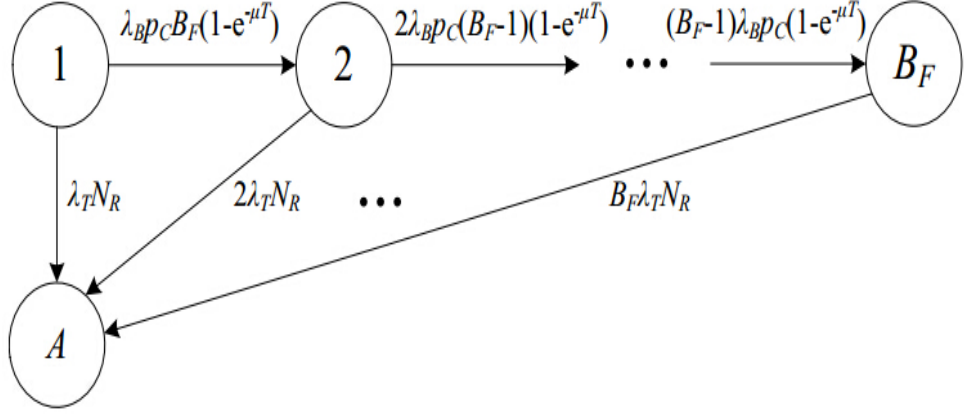


Figure. 5: Continuous time absorbing Markov chain model.

Let $S_i, i = 1, 2, \dots, N_B$, denote the sojourn time times in corresponding states and let $S_{i,i+1}$ and $S_{i,N+1}$ denote the sojourn time in the state i given that the chain leaves for state $i+1$ and $N+1$, respectively. We see that the chain may get absorbed from any state $i, i = 1, 2, \dots, N_N$. Thus, we have

$$E[e^{-\nu T}] = \sum_{i=1}^{N_B} E[e^{-\nu \sum_{k=1}^i S_k} | S(t) = i] P_r\{S(t) = i\}. \quad (3.12)$$

implying that we need to get conditional LSTs $E[e^{-\nu \sum_{k=1}^i S_k} | S(t) = i]$ and $P_r\{S(t) = i\}, i = 1, 2, \dots, N_B$ to get unconditional LST of the message delivery delay.

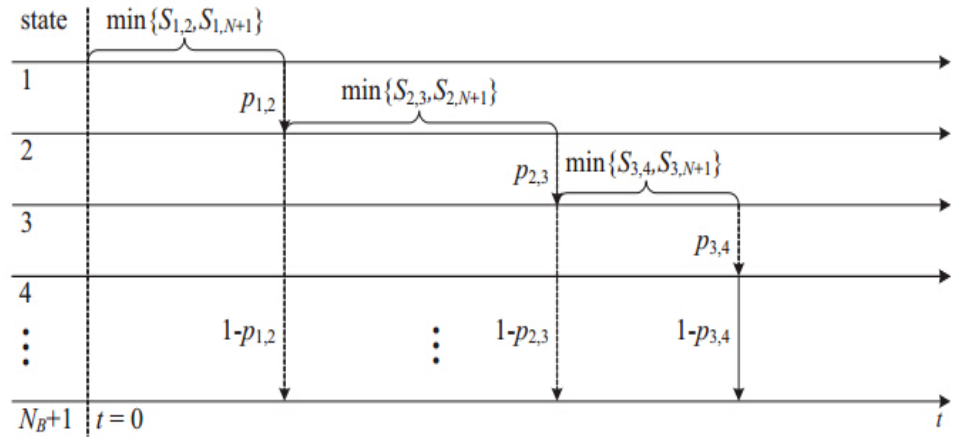


Figure. 6: Time diagram of the absorbing Markov model.

Note that in (3.12) $P_r\{S(t) = i\}$ is interpreted as the probability of absorbing from the state i , i.e. $P_r\{S(t) = i | S(t + \Delta t) = N_B + 1\}$ as $\Delta t \rightarrow 1$. In order for this event to happen the chain must first reach this state and then move to the state $N_B + 1$ without getting to the state $i + 1$. Due to simplicity of the model this probability can be estimated directly without resorting to numerical calculations. The event of absorbing from the state i happens only when the chain did not absorb in proceeding states $i = 1, 2, \dots, i - 1$, i.e. there is only one trajectory to take into account. Let $p_{i,i+1}$ and p_{i,N_B+1} denote the probability of going from i to $i + 1$ and from i to $N_B + 1$ at the moment of state change. Fig. 6 illustrates these quantities showing the time behavior of the model where the chain absorbs from the state 3. Since the index of two exponentially distributed random variables, X_1 and X_2 , with parameters λ_1 and λ_2 , that attains the minimum (the corresponding state change happens in our model) is given by

$$P_r\{X_1 = \min\{X_1, X_2\}\} = \frac{\lambda_1}{\lambda_1 + \lambda_2}, \quad (3.13)$$

we have

$$p_{i,i+1} = \frac{\lambda_T N_T}{p\lambda_B(N_B - i) + \lambda_T N_T}, p_{i,N_B+1} = \frac{p\lambda_B(N_B - i)}{p\lambda_B(N_B - i) + \lambda_T N_T} \quad (3.14)$$

such that $p_{i,i+1} + p_{i,N_B+1} = 1$.

The probability that the chain absorbs from the state i is then

$$P_r\{S(t) = i\} = p_{i,N_B+1} \prod_{k=1}^{j-1} p_{k,k+1} \quad (3.15)$$

The last thing left is to determine the conditional LSTs in (3.12). In order to get it we need to get the distribution of time the model spends in state $j, j = 1, 2, \dots, i - 1$, before entering the next state $k + 1$ as well as the distribution of time it spends in the state i before absorbing in the state $N_B + 1$. Recalling the definition of $S_i, S_{i,N_B+1}, i = 1, 2, \dots, N_B$ we observe that the distribution of the jumping point is given by $S_i = \min(S_{i,i+1}, S_{i,N_B+1})$ is also exponentially distributed with parameter $(\lambda_{i,i+1} + \lambda_{i,N_B+1})$ provided in (3.8), see Fig. 6. Further, we also see that due to the memoryless properties of the exponential distribution for any state i we have

$$P_r\{S_{i,i+1} < t | S_{i,i+1} < S_{i,N_B+1}\} = P_r\{S_{i,N_B+1} < t | S_{i,N_B+1} < S_{i,i+1}\} \quad (3.16)$$

and both are equal to

$$P_r S_i \leq t = 1 - e^{-\lambda_i^* t}, \lambda_i^* = \lambda_{i,i+1} + \lambda_{i,N_B+1} \quad (3.17)$$

The latter result implies that conditional LSTs can be written as

$$E[e^{-\nu \sum_{k=1}^i S_k} | S(t) = i] = E[e^{-\nu [(\sum_{k=1}^i S_{i,i+1}) + S_{i,N_B+1}]} | S(t) = i] \quad (3.18)$$

where

$$S_{j,j+1} < S_{j,N_B+1}, j = 1, 2, \dots, i-1, S_{i,i+1} > S_{i,N_B+1} \quad (3.19)$$

Combining exponentiality of (3.17) with (3.18) we get

$$E[e^{-\nu \sum_{k=1}^i S_k} | S(t) = i] = \prod_{k=1}^{i-1} E[e^{-\nu S_k}] = \prod_{k=1}^{i-1} E\left[\frac{\lambda_k^*}{\lambda_k^* + k}\right] \quad (3.20)$$

Unconditioning using (3.15) we get LST of the message delivery delay

$$L_T(\nu) = \sum_{i=1}^{N_B} \left(p_{i,N_B+1} \prod_{k=1}^{j-1} p_{k,k+1} \right) \prod_{k=1}^{i-1} p_{k,k+1} \frac{\lambda_k^*}{\lambda_k^* + k} \quad (3.21)$$

Differentiating (21) and setting $\nu = 0$ we get the mean delivery delay.

3.2.5 Multiple transmitter-receiver model

Consider now multiple transmitter-receiver case assuming that all transmitters starts communicating at a certain time $t = 0$ releasing B_E bacteria each. We concentrate on a certain randomly chosen pair and consider the rest of transmissions as a single interference process emitting $(N-1)B_E$ bacteria at $t = 0$. This pair is called tagged in what follows. The important thing for the proposed model is that the inter-conjugation time is assumed to be significantly larger than the information delivery time. It means that a certain free bacteria can only conjugate at most once during the modeling time. This assumption appears to be realistic but could be relaxed when needed.

The resulting model is a direct extension of the model for a single pair of transmitter-receiver. The state transition diagram is shown in Fig. 7, describing the case of B_E emitted bacteria by each of N sources. The transitions in each row corresponds to increasing the population of bacteria of the tagged source due to conjugation process. Similarly, transitions in each column corresponds to the the increase of the population of the interfering process. Finally, transitions to the absorbing state is possible from any state of the chain.

Transition probabilities are

$$\begin{aligned}
 (i, j) &\rightarrow (i-1, j+1) : ij\lambda_B p_C (1 - e^{-\mu T}) \\
 (i, j) &\rightarrow (i, j-1) : j(B_E N + B_F - i + B_E)\lambda_B p_C (1 - e^{-\mu T}) \\
 (i, i) &\rightarrow A : i\lambda_R
 \end{aligned} \tag{3.22}$$

where λ_B is IPT rate between bacteria, λ_R is the meeting rate between bacterium and receiver, p_C is the conjugation probability, μ is the rate of environment shaking process.

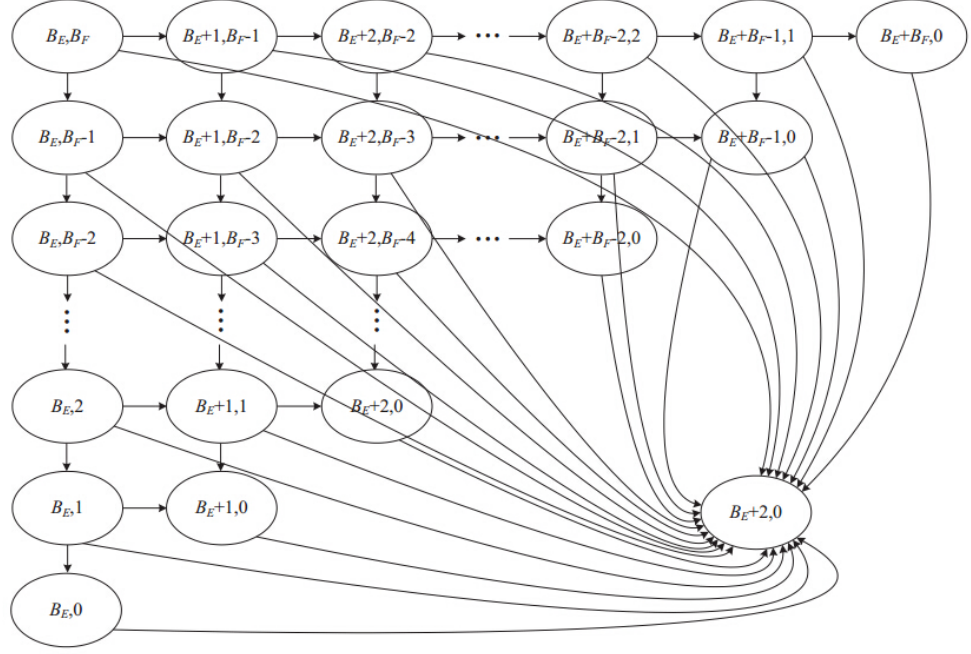


Figure. 7: The model for multiple transmitter-receiver pairs.

Note that the transitions rates provided in (3.8) describe the case of a single receiver. Instead of a single receiver the model can be extended to the case of multiple receivers for a single transmitter. The only modifications needed is to changes transitions to the absorbing state. We get the system with multiple receivers for a single source using

$$(i, j) \rightarrow A : iN_R\lambda_R, \tag{3.23}$$

where N_R is the number of receivers.

3.3 Core code in matlab

We have shown that multiple transmitter-receiver scenarios are complicate, for the same reason we have a large number of diagrams can be drawn. In total more than 100 plots for different input parameters are there, we do not want to generate all

these plots at the same time which is not efficient. So we have some fancy code here to prevent this feckless situation from happening, instead we only draw several diagrams which we are interested in.

In the following code we find out the targeted data from the data structure where all the data that we have collected are saved:

```

1 for val = value
2     if val ~= 0
3         elseif type == 2%emitted bac
4             pos = find(emitBacArr == val)
5             [rowOfSameEmtBac, colOfSameEmtBac] = find(
6                 location(target,3) == pos,row/lenEmtBacArr);
7             target = target(rowOfSameEmtBac);
8             row = length(rowOfSameEmtBac);
9         elseif type == 1%free bac
10            pos = find(freeBacArr == val)
11            [rowOfSameFreeBac, colOfSameFreeBac] = find(
12                location(:,2) == pos,row/lenFreeBacArr);
13            target = rowOfSameFreeBac;
14            row = length(rowOfSameFreeBac);
15        end
16    end
17    type = type + 1;
18 end
19 rows = target;

```


4. RESULTS AND DISCUSSION

In this chapter we provide numerical results obtained by solving the models formulated above. We will start addressing the case of a single transmitter-receiver pair first observing the effects of the number of free and emitted bacteria and highlighting the role of conjugation. Then, we proceed analyzing the multiple transmitter-receiver case. In both cases the performance metrics of interest are CDFs and 0,95-quantile of delivery time as well the the mean delivery time.

4.1 Single transmitter-receiver pair

4.1.1 Delivery time distribution

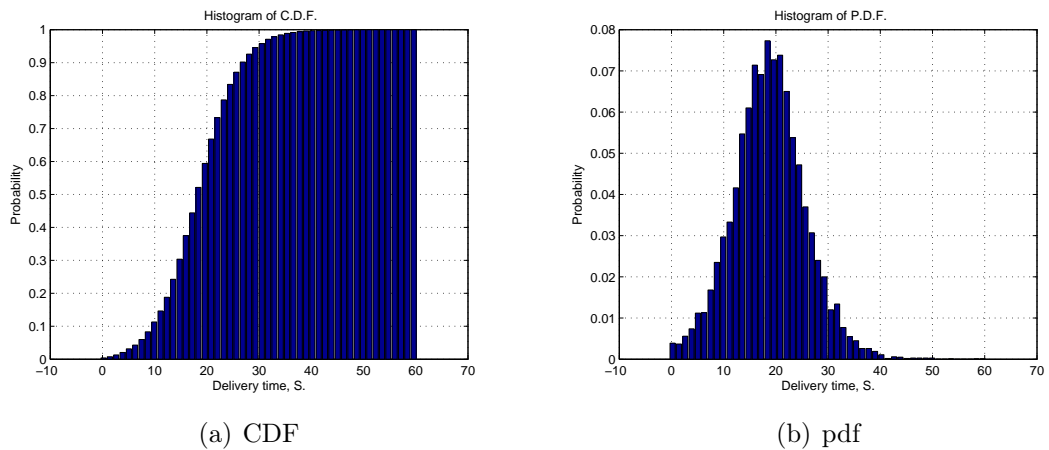


Figure. 8: Single transmitter-receiver pair, 1 emitted/1000 free bacteria, $1000\mu m$ compartment size.

In Fig. 8 we show the CDF and pdf diagrams of single transmitter-receiver pair, 1 emitted bacterium, 1000 free bacteria with $1000\mu m$ compartment size, this is the first step that we conduct this simulation and the results are quite accessible, we can easily see that the delivery time follows exponential distribution.

It is a good beginning that our simulation starts from single transmitter-receiver pair and some fixed parameters, nevertheless that's not our destination, we are going to introduce various compartment size, number of free bacteria, number of emitted bacteria to the system, then we study how those parameters impact on

the distribution of the delivery time. In order to analysis the behaviors of different parameters we present plots of CDF of delivery time, mean delivery time and 0.95 quantile of delivery time such that we can dig those data in more detail and try to find their relations.

Fig. 9 illustrates CDFs of delivery time for few selected input parameters including the number if free bacteria, the number of emitted bacteria and compartment size. First, as one may observe for small number of free bacteria and/or small number of emitted bacteria the CDF has clear exponential behavior. This is a direct consequence of the model predicting exponential meeting and inter-passage times. When the number of free and/or emitted bacteria increase the distribution starts to have a mode (not shown). These illustration are also telling with respect to the effect of conjugation. In particular, both the increase in the number of emitted bacteria and free ones improves the performance in terms of the delivery time. However, their effect is not equal. Below we highlight this effect in more detail using mean and quantiles of delivery time as metrics of interest.

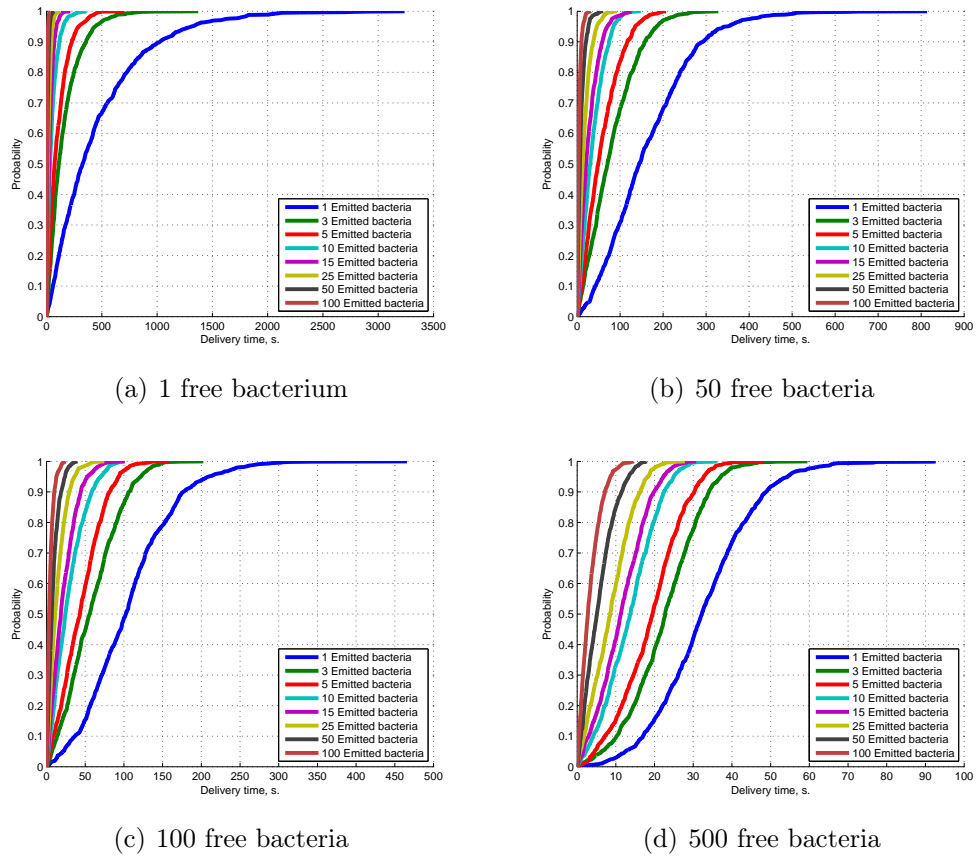
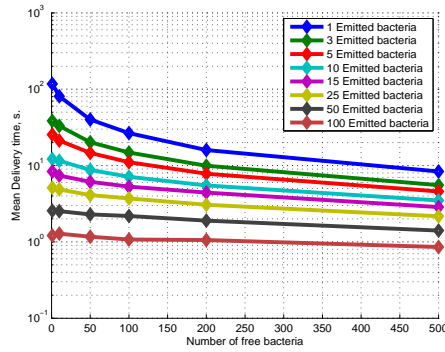


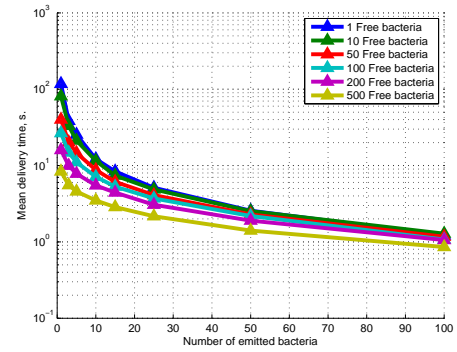
Figure. 9: CDFs of delivery time for different input parameters.

4.1.2 Mean delivery time

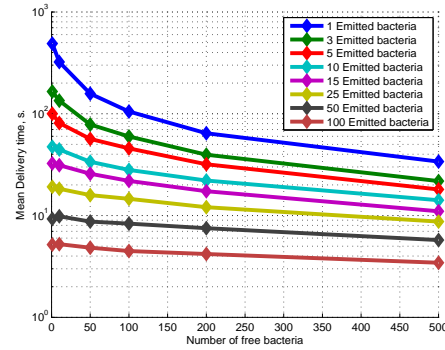
Fig. 10 demonstrates the mean delivery time for two compartment sizes, 500 and 1000 μm . As one may observe even the slight increase from 1 to 10 free bacteria cases a significant reduction in the mean delivery time. Moreover, the magnitude of the reduction increases as the compartment size gets bigger. In particular, for one emitted bacteria increasing the number of free bacteria from 1 to 10 the mean delivery time decreases from 118s. to 87s (by 26%). The corresponding decrease for 1000m compartment size is from 470s. to 324s (33%). In other words, for smaller compartment the increase in the number of free bacteria provides smaller gains compared to larger ones. The same trend is observed for larger compartment sizes and larger values of emitted bacteria.



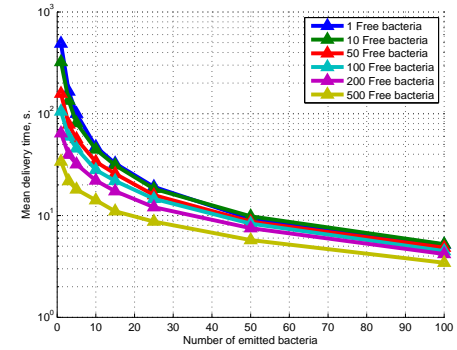
(a) 500 μm size, x: free bacteria



(b) 500 μm size, x: emitted bacteria



(c) 1000 μm size, x: free bacteria



(d) 1000 μm size, x: emitted bacteria

Figure 10: Mean delivery time for different compartment sizes.

It is important to note that the positive effect of the number of emitted bacteria is *quantitatively stronger* compared to the number of free bacteria. For example, increasing the number of emitted bacteria from one to two the decrease in the mean delivery time is from 118s. to just 38s. for 500 μm (by 78%) compartment and from 470s. to 164s. (by 77%) for 1000 μm compartment. This behavior is

explained by the fact that the distribution of time, $F(t)$, required to reach an empty bacteria is similar to that required to deliver the information to the receiver. Thus, for exponential meeting and inter-passage times and independent movement, for two emitted bacteria the distribution of time to reach the receiver is given by the minimum of two exponential distributions with the same parameter which is again exponential with mean $1/2\lambda$. For one emitted bacterium and one free bacterium the mean delivery time is provided by two components: the time to reach the receiver given that the emitted bacteria reaches it first, $2/\lambda$ with probability $1/2$ and the time to reach the receiver given that conjugation happen first. The latter terms is the mean of Erlang distribution with mean $2/\lambda$ and this case occurs with probability $1/2$. Thus, using the law of total probability we have $(1/4\lambda + 1/\lambda)$. The latter is bigger for any $\lambda > 1$.

Note that the above-mentioned data also show that the positive effect is the number of emitted bacteria is *independent of compartment size* which is different from the effect of the number of free bacteria. This is however, true for relative gain, as the absolute numbers are obviously bigger for larger compartment size.

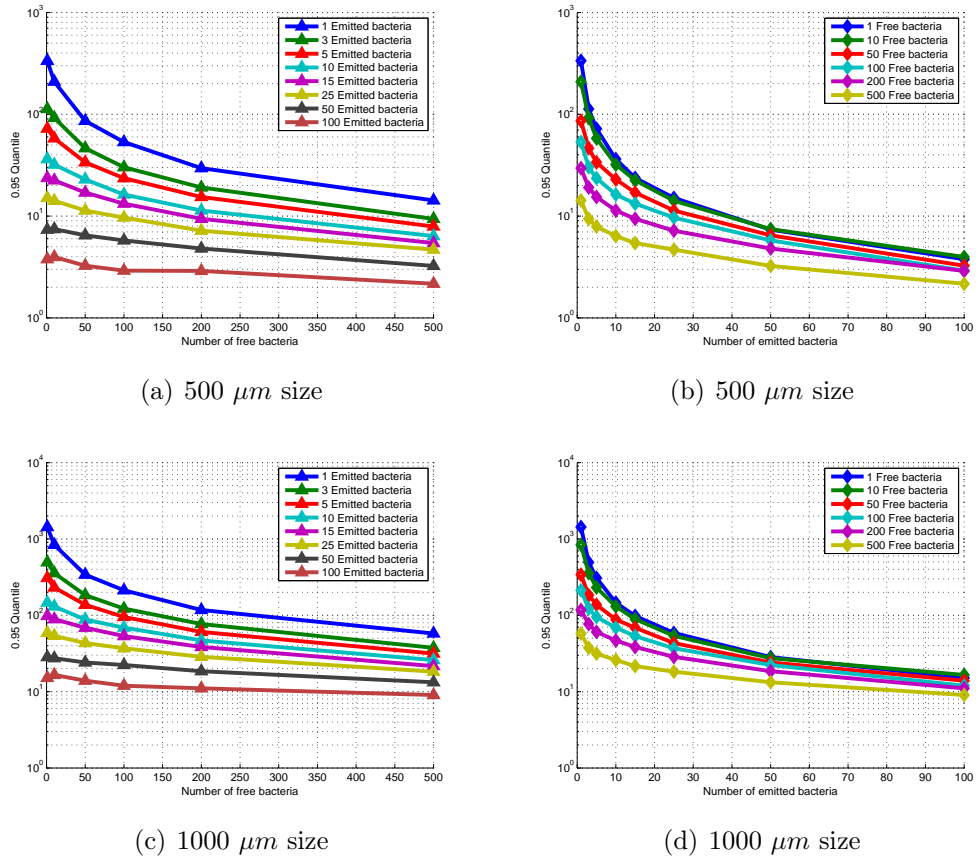


Figure 11: 0.95 quantile of delivery time for different compartment sizes.

4.1.3 0.95 quantile

Let us now concentrate the metric pertaining to reliability of the message delivery by discussing 0.95 quantile of the delivery time distribution demonstrated in Fig. 11. Recall that the x -quantile of a distribution provides the value y such that the accumulated probability up to this point is at most x . Interpreting it in terms of the problem in hand, 0.95 quantile is the amount of time up to which in 95 out of 100 times the information is delivered to the receiver. As one may notice in Fig. 11 the trends and conclusion highlighted for mean above remain valid for quantiles as well. The presented 0.95 quantile data can be used to choose the required quantity of emitted/free bacteria such that the delivery time is upper bounded by a certain value.

4.2 Multiple transmitter-receiver pairs

4.2.1 Delivery time distribution

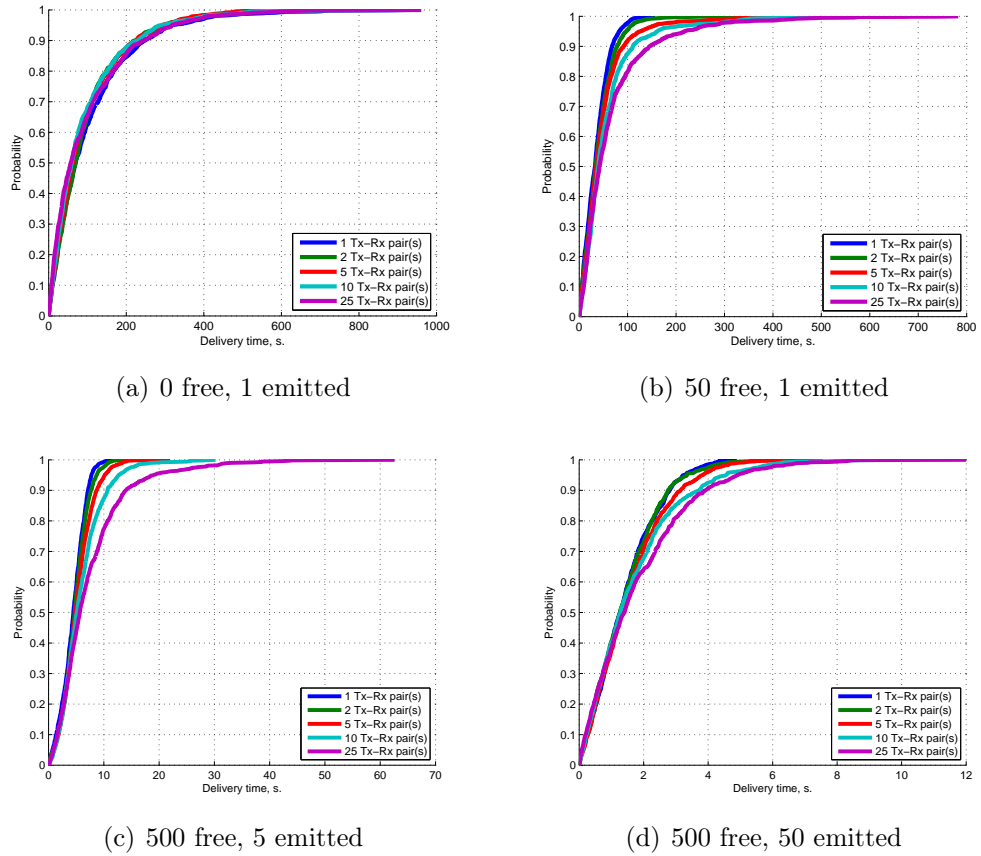


Figure 12: CDFs of delivery time for different input parameters.

Consider now the effect of multiple transmitter-receiver pairs. First, to validate the proposed model we refer to Fig. 12 illustrating CDFs of the delivery time for different numbers of transmitter-receiver pairs, one emitted bacteria, zero free bacteria and compartment size of $500\mu m$. As expected, all CDFs coincide with each other (up to the statistical error) implying that the model indeed estimates performance statistics of a single pair in presence of other pairs acting as interference. Also, we note that comparing performance using CDFs is extremely inconvenient and we immediately proceed with means and quantile. of mean delivery time.

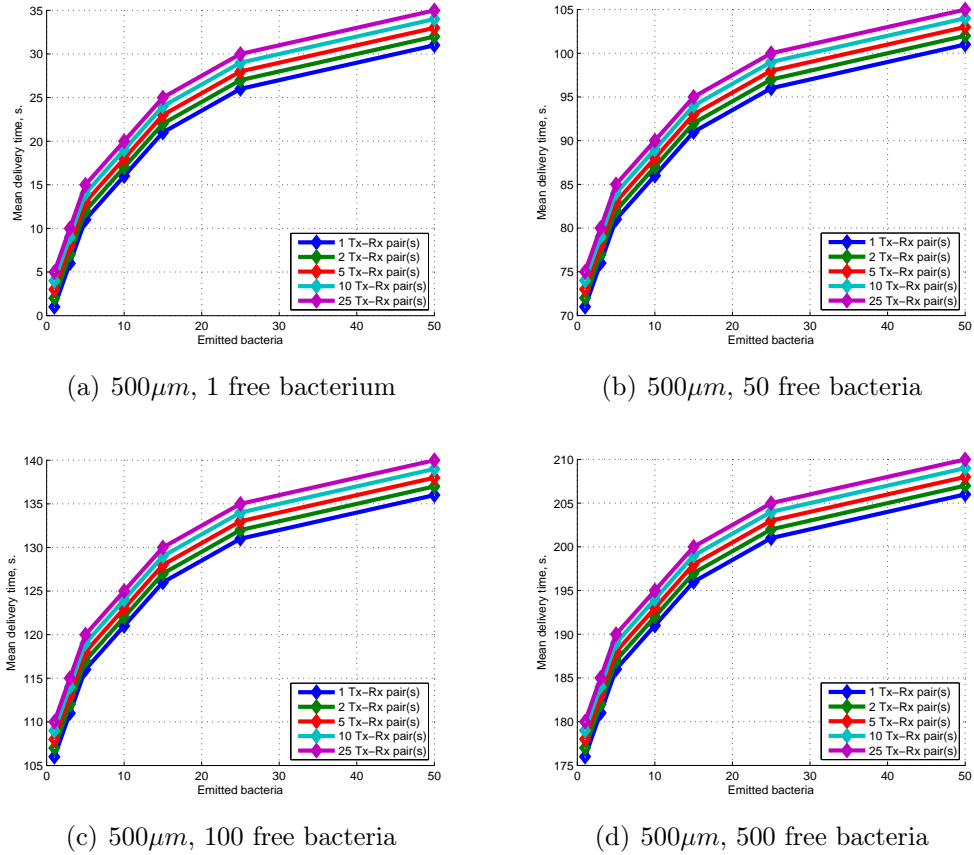


Figure 13: Mean delivery time for different number of transmitter-receiver pairs.

4.2.2 Mean delivery time

Mean delivery time for different number of transmitter-receiver pairs, number of emitted and free bacteria is illustrated in Fig. 13, where the compartment size is fixed to $500\mu m$. As one may observe. there are obvious dependencies between the mean time and input parameters. In particular, the positive effect of the number of emitted and free bacteria is preserved while the increase in the number of transmitter-receiver pairs leads to the corresponding quantitative increase in the

mean delivery time. Note that these trends are preserved for higher number of free bacteria including 100, 200 and 500. For practice it means that we shouldn't expect any drastic performance degradation when more than one transmitter-receiver pair is expected to exchange information at the *same time*.

The timeliness of information exchange is of special interest. Indeed, the model analyzed in this thesis presumes that all the transmitters start to emit bacteria at the same time. However, if one or more pairs start the transmission process a bit early they get additional advantage in terms of the number of free bacteria available as the transmission process evolves. Correspondingly, those pairs starting later may face the situation of free bacteria already occupied by information of other pairs. The performance experienced by these pairs can still be evaluated using the proposed model by appropriately setting the number of free bacteria available for them at the beginning of the transmission process.

4.2.3 0.95 quantile

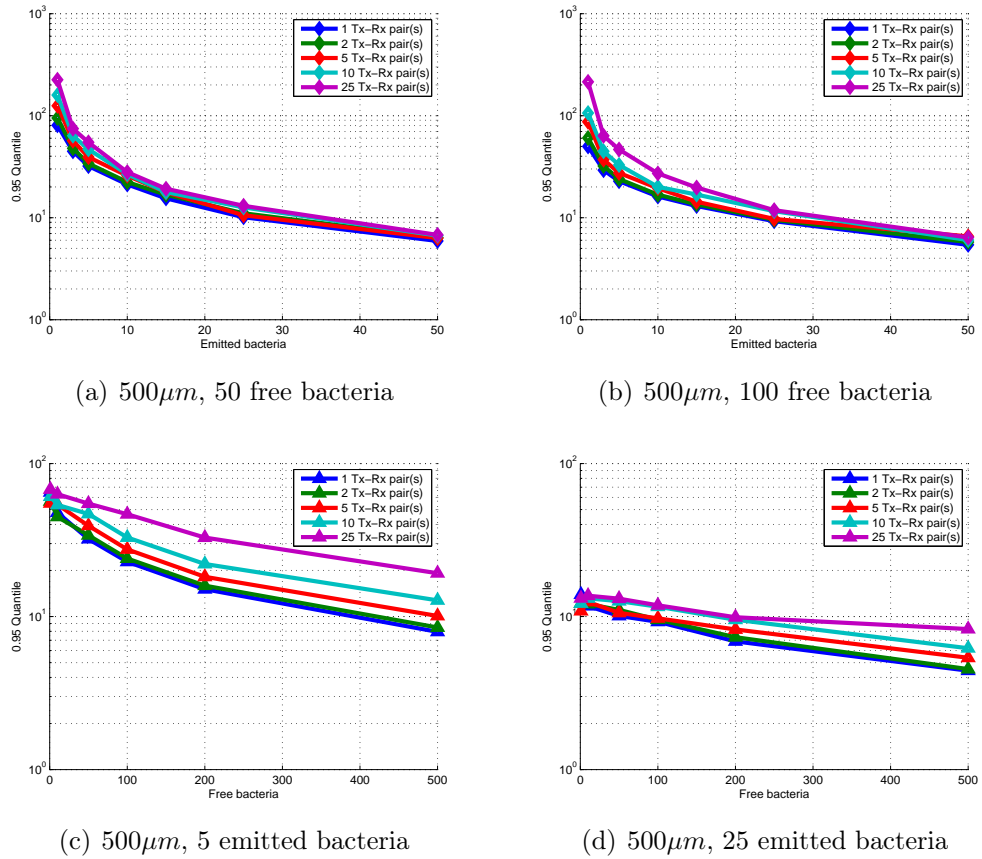


Figure 14: 0.95 quantile of delivery time for different number of transmitter-receiver pairs.

0.95 quantiles of the delivery time for different numbers of transmitter-receiver pairs, emitted and free bacteria and compartment size of $500\mu m$ are shown in Fig. 14. The provided data has no special interesting trends to discuss and characterized by expected increase in the 0.95 quantiles with the number of transmitter-receiver pairs increasing. The effect of the number of emitted bacteria is exponential and more profound when the number of free bacteria increasing as well. These quantile data can be used for determining the number of emitted and free bacteria in a compartment of a certain size such that the higher bound on delivery delay is satisfied [9].

5. CONCLUSIONS

In this work the Markov models for delivery time estimation in bacterial networks have been developed. The models take into account the number of special features of such systems including (i) multiple emitted bacteria carrying the same information between single transmitter-receiver pair, (ii) conjugation effect explicitly accounting for the number of free bacteria available in the environment and (iii) multiple transmitter-receiver pairs performing transmitting information simultaneously. The model for single transmitter-receiver pair allows for rudimentary analytical analysis including mean and distribution of the information delivery time. Although the Markovian structure is preserved in the model for multiple transmitter-receiver pairs the state-space explodes allowing for analytical treatment in special cases only. In this thesis, Monte-Carlo simulations were used to determine quantities of interest.

We performed a systematic investigation of the effect of input parameters on the delivery performance of the system. The following are the main findings: (i) the number of emitted bacteria and the number of free ones provides noticeable quantitative gains in terms of mean delivery time, (ii) the gain provided by the number of emitted bacteria is significantly stronger compared to the effect of free bacteria, (iii) the relative gain of free bacteria strongly depends on the compartment size (iv) the relative gain associated with the number of emitted bacteria is independent of the compartment size. The effect of multiple transmitter-receiver pairs is straightforward - the increase in their number leads to the corresponding increase in the mean delivery time. Importantly, we provided the results for the 0.95 quantiles of delivery time that can be used in practice to decide upon the number of emitted and free bacteria such that a certain delivery time guaranty is satisfied [9].

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